Supplementary Table 1: Growth analysis comparison of parental wild-type FMDVs and thermostable FMDVs. BHK-21 cells were infected with the respective virus (0.1 m.o.i.) and samples analysed at 8 h post-infection by plaque assay to determine viral titres. Similar results were obtained from three individual experiments.

Serotype		Titre (pfu/ml)
O1M	Wild-type	1.1×10^5
	VP2 S93Y	2×10^4
	VP2 S93F	1×10^4
	VP2 S93W	1×10^4
	VP2 S97Q	9×10^4
	VP2 Y98F	2×10^4
SAT2	Wild-type	3.2×10^6
	VP2 S93H	2.4×10^7
	VP2 S93Y	1.7×10^5
	VP2 S93W	2.8×10^6

Supplementary Table 2: Thermostability of infectious O1M and inactivated SAT2 viruses measured in triplicates by fluorescence assay at pH 7.5.

		Tm (n=3) ˚C
O1M	Wildtype	52.0 ± 0.0
	93H	51.5 ± 0.0
	93Y	53.4 ± 0.2
	93F	53.3 ± 0.3
	93W	52.0 ± 0.1
	97Q	53.8 ± 0.3
	98F	53.8 ± 0.3
SAT2	Wildtype	47.1 ± 0.2
	93H	51.2 ± 0.3
	93Y	53.4 ± 0.3
	93W	47.2 ± 0.1

Supplementary Table 3: CryoEM data collection and refinement statistics

CryoEM Detector	**K2 Summit**	
Data set	**O1M VP2 S93Y**	**SAT2 VP2 S93Y**
Particles	8267	8156
Pixel Size (Å)	1.35	1.35
Defocus Range (μm)	0.8-2.5	0.8-2.5
Voltage (kV)	300	300
Electron Dose (e^{-} $Å^{-2}$)	18	18
Resolution (Å)	3.2	3.5
Map Sharpening B-factor ($Å^2$)	-113.8	-121.4
Model Refinement		
Fo-Fc Correlation	0.85	0.87
Protein atoms	5159	5246
R.m.s.d., bonds (Å)	0.01	0.01
R.m.s.d., angles (°)	1.01	0.98
Clashscore, all atoms (percentile)	6.21 (90^{th})	8.75 (78^{th})
Rotamer Outliers (%)	0.0	0.0
Ramachandran outliers (%)	1.38	2.29
MolProbity score (percentile)	1.77 (87^{th})	1.91(80^{th})

Supplementary Note

Molecular dynamics simulation methods

Model preparation for MD simulations

Model preparation and visual analysis used COOT[1], PyMOL (http://www.pymol.org/) and custom scripts for generating symmetry-related molecules and detecting steric clashes. Several FMDV capsid structures have been determined[2-6], some of which were solved some time ago and/or were only at moderate resolutions, so careful preparation was required to make suitable starting models. Wild-type models were constructed for FMDV strains O1BFS (PDB ID: 1BBT)[7], A22 (4GH4)[3] and SAT1 (PDB ID: 2WZR)[6] by expanding crystallographic and non-crystallographic symmetry to generate a dimer interface. Water molecules were added where possible.

Preparation of truncated models for simulation

Program SELECT3 (RME, unpublished) prepared truncated structures and generated dummy atoms along the interface of interest (see below). SELECT3 reads in two PDB files describing the molecules on either side of the inter-pentameric interface and a third file describing crystallographic waters. First, all inter-pentamer interactions (inter-atomic distances across the interface < 4 Å) were detected and a list of "dummy" atoms placed at the midpoint of each interaction was created. A truncated model was then generated which contained all atoms within 13 Å of any interfacial dummy atom. Isolated amino acids were filtered out and the remaining selected residues were written out in AMBER-compatible PDB format ready for simulation. The water atom list was similarly truncated and written out in PDB format.

Design and construction of candidate mutants

For each truncated model, amino acids contributing to the inter-pentameric interface in the vicinity of the capsid 2-fold symmetry axis were identified using the EBI-PISA web-server. Visual analysis of the interactions made by these residues (using COOT and PyMOL) particularly by reference to more stable picornaviruses and comparison of the electrostatics facilitated the rational design of a panel of putative stabilizing mutations that might improve hydrophobic and/or electrostatic interactions across the interface (**Table S1**). These were incorporated using COOT[1] by simple mutation and energy minimization. Clashing water molecules were deleted.

MD simulation protocol

All simulations used AMBER10[8] (University of California, 2008). The truncated models for the wild-type and mutants were simulated for 1.55 ns, using the explicit solvent model with long-range electrostatic interactions handled by the periodic boundary condition and Ewald sums using a spacing of 1Å. Prior to simulation, hydrogen atoms were placed and the surface charge was neutralised by adding counter ions. The system was then solvated into a rectangular box of TIP3P[9] water molecules such that no atoms in the starting model were less than 10 Å from the edge of the water box. The system was minimised in three steps. First, the solvent was minimised, keeping all non-water atoms effectively fixed (restraint weight 500 kcal/mol). Secondly, the solvent was further minimised along with counter ions, keeping all protein (and dummy) atoms effectively fixed. Finally, all atoms except dummy atoms were minimised under the influence of the dummy-atom derived restraints (restraint weight 50 kcal/mol for atoms >10 Å from any dummy atom). This was followed by gradual heating from 0 K to 310 K over 50 ps. The temperature was controlled by Langevin dynamics with a collision frequency, γ, of 2.0 ps^{-1}. Next the system was run for 550 ps during which the

structures equilibrated, as judged by positional shifts and overall energy terms (data not shown), and this was followed by a 1 ns production run. The non-bonded distance cut-off was 8 Å at all stages and the SHAKE algorithm was used to constrain the lengths of bonds involving hydrogen atoms. All dynamic simulations were performed at 310 K in a constant volume (NVT) ensemble with a time-step of 2 fs and dummy-atom derived restraints. The simulations are at neutral pH.

Estimation of degree of stabilization (*ΔΔG*)

The calculation of the binding free energy, *ΔG*, between adjacent protomers used the published Molecular Mechanics Poisson Boltzmann, MM-PBSA, approach embodied in the *mmpbsa* module of AMBER[10, 11]. In MM-PBSA, the binding free energy between two protomers to form a complex is calculated as:

$$\Delta G = \Delta H - T\Delta S \cong \Delta E_{MM} + \Delta G_{sol} - T\Delta S \quad (1)$$

$$\Delta E_{MM} = \Delta E_{internal} + \Delta E_{electrostatics} + \Delta E_{vdw} \quad (2)$$

$$\Delta G_{sol} = \Delta G_{polar} + \Delta G_{non-polar} \quad (3)$$

ΔE_{MM} is the internal energy of the system and it is calculated using the gas phase energies from MM, ΔG_{sol} is the solvation free energy and $-T\Delta S$ represents the entropy change upon binding. ΔE_{MM} is the sum of bonded energies ($\Delta E_{internal}$) which includes energies from bond-stretching, angle-bending and torsion energies; other components include electrostatic interactions ($\Delta E_{electrostatics}$) from charged atoms described by the Coulomb potential as shown in equation 2 above and energies from van der Waals (vdw) interactions (ΔE_{vdw}). ΔG_{sol} is the sum of polar (ΔG_{polar}) electrostatics and non-polar ($\Delta G_{nonpolar}$) contribution to the solvation free energy. The polar contribution is calculated using the Poisson-Bolzmann (PB) method

and the non-polar contribution is estimated by solvent accessible surface area (SASA)[11, 12]. The entropy change $-T\Delta S$ is calculated using normal mode analysis. All these calculations are iteratively performed on a set of snapshots taken from MD trajectories to get the statistically significant ΔG_{bind} and an estimate of the error.

To reduce the noise and cancel errors in simulations, binding energy was calculated from a single trajectory of MD simulation. A snapshot taken at every 10 ps during the final 1 ns of simulation was used to calculate each free energy component in the above equations and the binding free energy was represented as the mean of 100 snapshots. Finally, the difference in binding free energy, $\Delta\Delta G$, between candidate mutant models and the parent wild-type model was calculated using (4) to assess the stability of mutants.

$$\Delta\Delta G = \Delta G_{wt} - \Delta G_{mut} \qquad (4)$$

1. Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallographica Section D* **60**, 2126-2132 (2004).
2. Acharya, R. et al. The three-dimensional structure of foot-and-mouth disease virus at 2.9 A resolution. *Nature* **337**, 709-716 (1989).
3. Curry, S. et al. Perturbations in the surface structure of A22 Iraq foot-and-mouth disease virus accompanying coupled changes in host cell specificity and antigenicity. *Structure* **4**, 135-145 (1996).
4. Curry, S. et al. Dissecting the roles of VP0 cleavage and RNA packaging in picornavirus capsid stabilization: the structure of empty capsids of foot-and-mouth disease virus. *J Virol* **71**, 9743-9752 (1997).
5. Lea, S. et al. The structure and antigenicity of a type C foot-and-mouth disease virus. *Structure* **2**, 123-139 (1994).
6. Reeve, R. et al. Sequence-Based Prediction for Vaccine Strain Selection and Identification of Antigenic Variability in Foot-and-Mouth Disease Virus. *PLoS Comput Biol* **6**, e1001027 (2010).
7. Fry, E., Acharya, R. & Stuart, D. Methods used in the structure determination of foot-and-mouth disease virus. *Acta Crystallogr A* **49**, 45-55 (1993).
8. Case, D.A. et al. The Amber biomolecular simulation programs. *J Comput Chem* **26**, 1668-1688 (2005).

9. Price, D.J. & Brooks, C.L., 3rd A modified TIP3P water potential for simulation with Ewald summation. *J Chem Phys* **121**, 10096-10103 (2004).
10. Case, D.A., T.A. Darden, T.E. Cheatham, III, C.L. Simmerling, J. Wang, R.E. Duke, R. Luo,, M. Crowley, R.C.W., W. Zhang, K.M. Merz, B.Wang, S. Hayik, A. Roitberg, G. Seabra, I., Kolossváry, K.F.W., F. Paesani, J. Vanicek, X.Wu, S.R. Brozell, T. Steinbrecher, H. Gohlke,, L. Yang, C.T., J. Mongan, V. Hornak, G. Cui, D.H. Mathews, M.G. Seetin, C. Sagui, V. Babin, & Kollman, a.P.A. (University of California, AMBER 10; 2008).
11. Kollman, P.A. et al. Calculating Structures and Free Energies of Complex Molecules: Combining Molecular Mechanics and Continuum Models. *Accounts of Chemical Research* **33**, 889-897 (2000).
12. Hou, T., Wang, J., Li, Y. & Wang, W. Assessing the performance of the MM/PBSA and MM/GBSA methods. 1. The accuracy of binding free energy calculations based on molecular dynamics simulations. *J Chem Inf Model* **51**, 69-82 (2011).